Crystal Quality – The long and the short of it.

Edward Snell

What is crystal quality?

- Crystal quality is an assessment or measurement of properties of a crystal or data from the crystal.
- Crystal quality is used to determine which crystal to focus on for data collection or to understand the effect of experimental variables on the growth.
- Crystal quality can be a quantitative or qualitative term.
- The term “crystal quality” is ambiguous. It can imply different meanings to different fields.

- There are many experimental factors to consider when comparing crystal quality metrics.

Crystal quality and structural crystallography

- A high quality crystal for a structural crystallographer is a crystal that provides answers to structural questions with minimum experimental effort.
- This translates to a crystal that;
  - diffracts to a high resolution
  - provides clear signal to noise
  - is radiation insensitive
  - results in a interpretable electron density map
- In terms of crystallographic metrics a high quality crystal;
  - provides data that shows significant signal to noise at or beyond a desired resolution, has good completeness, high redundancy and good agreement between symmetry equivalent observations

Crystal quality and physical crystallography

- Physical crystallography concerns:
  - the packing and order of molecules within the crystal
  - imperfections in the packing
  - growth of crystals
- In physical crystallography the structure of the molecule is not of prime interest.
- Physically, a high quality crystal is defined as one that has both good short-range and long-range order.
- This translates to a crystal that;
  - is high quality in a structural crystallography sense
  - has low mosaicity
  - is large in volume

Crystal growth

- Crystal growth is concerned with;
  - growing crystals
  - growing better crystals
  - growing the best crystals
- Crystal growth involves a vast range of variables that can be optimized to achieve the best crystals.
- Crystal quality measurements allow the best quality crystals to be determined and give feedback to optimizing and understanding the effect of variables on the growth process.
- Structural crystallography and physically crystallography combine to give useful metrics to define the best crystal.

Criteria used to describe crystal quality

- Visible
  - Clarity, number, dimensions, volume, morphology, reproducibility.
- Diffraction properties
  - Mosaicity, resolution, agreement between symmetry related reflections, cell parameters, completeness, thermal motion of atoms.
- Resulting structure
  - Agreement between model and data, agreement between unrefined data, agreement with structure and geometrical restraints, variability in atomic positions.

Visible properties

- Clarity
  - A good quality crystal lacks visible cracks and defects.
- Number
- Dimensions
  - Length to width ratios are important in terms of the diffraction cross section.
- Volume
  - Volume is also important in terms of diffraction cross section.
- Morphology
  - An indicator of a possible change in quality. Different morphology can indicate a change in space group and a consequent change in diffraction properties due to packing differences.
- Reproducibility
  - Reproducibility is a key requirement if the observations are meaningful for crystal growth studies.

	Independent of:						Use to compare:	
Metric	Data collection protocol	X-ray beam	Detector	Qualitative judgment	Structure solution		Within same sample	Across samples
Visible								
Clarity	Yes	Yes	Yes	No	Yes		Yes	No
Number	Yes	Yes	Yes	Yes	Yes		Yes	No
Dimensions	Yes	Yes	Yes	Yes	Yes		Yes	No
Volume	Yes	Yes	Yes	Yes	Yes		Yes	No
Morphology	Yes	Yes	Yes	Yes	Yes		Yes	No
Reproducibility	Yes	Yes	Yes	Yes	Yes		Yes	No

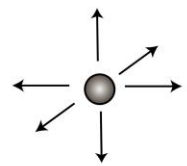
However beautiful crystals can have very poor diffraction characteristics and similarly bad looking crystals can have high quality diffraction characteristics.

The most important visible characteristic is reproducibility.

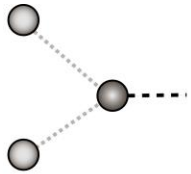
Diffraction properties

- Can be qualitative or quantitative.
- Can be divided properties caused by
  - Short range order
  - Long range order
- Can be further divided into factors that are
  - measured from single reflections
  - measured by a statistical sample of reflections
  - measured with a complete data sets.
- Diffraction properties are the best measurements of crystal quality as the ultimate use is for diffraction experiments.

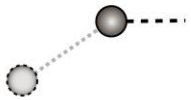
Short-range disorder (molecular scale)



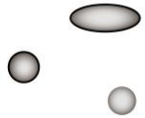
Atomic displacement



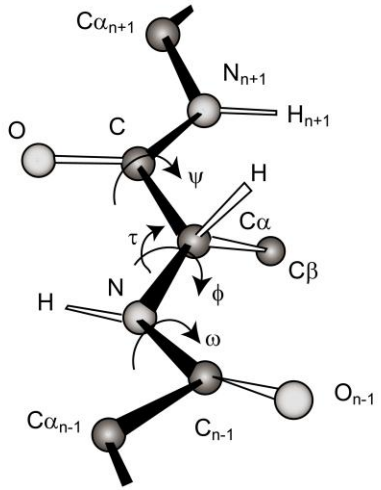
Multiple conformation



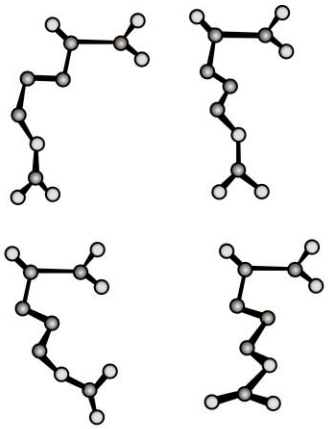
Partial occupancy



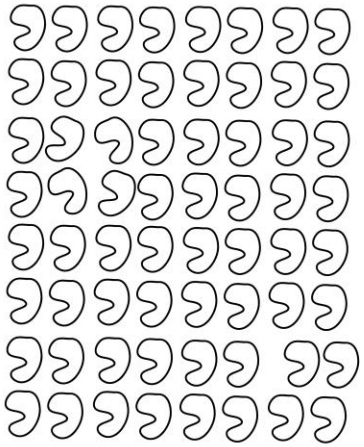
Waters



Main chain variation



Side chain variation

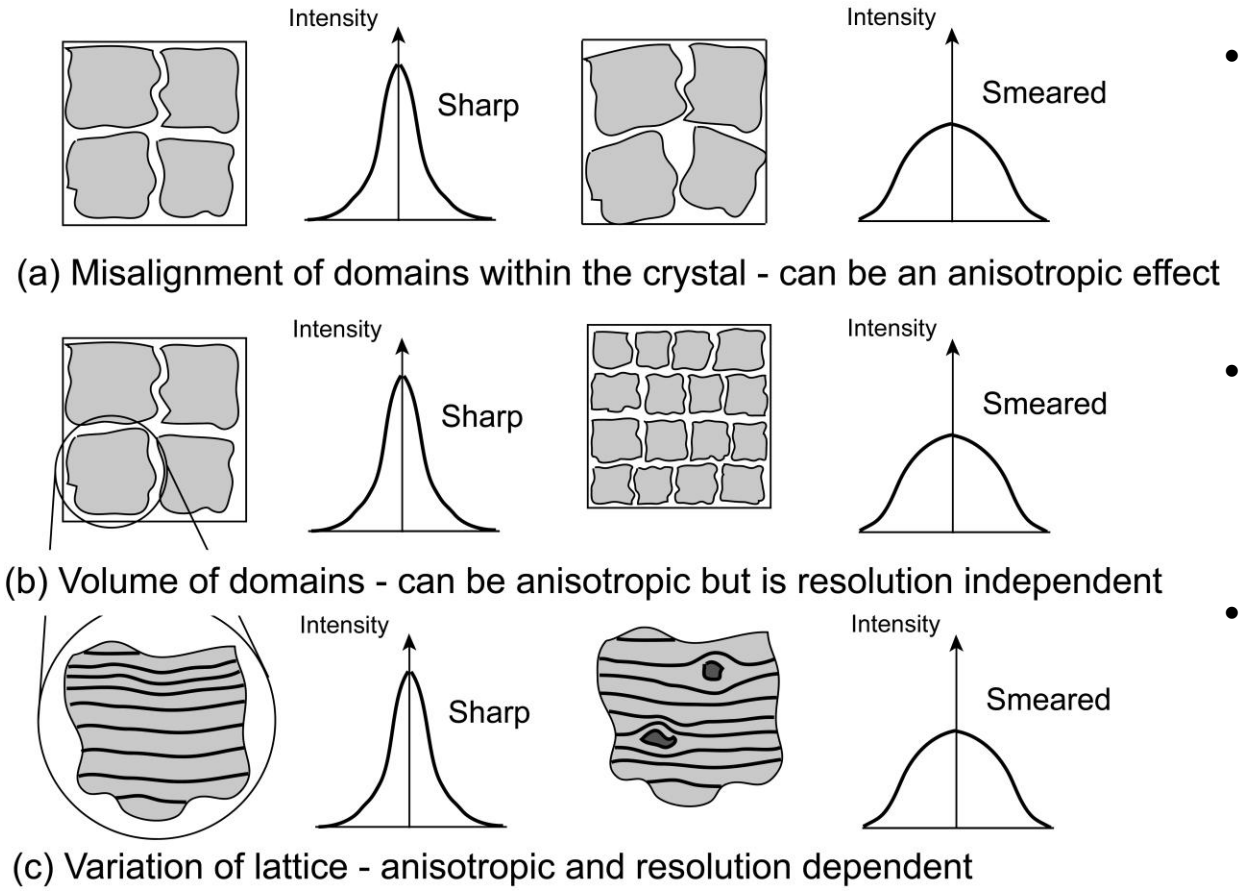


Inter-molecular packing

- Atomic displacement (thermal motion), i.e. B-factor.
- Multiple atom and side chain conformations.
- Partial occupancy.
- Ordered and disordered water.
- Main chain variation.
- Packing defects.

Diffraction data is a ‘snapshot’ of the average structure. Molecular scale deviations from that average reduce the quality of the diffraction data.

Long-range (crystal scale)

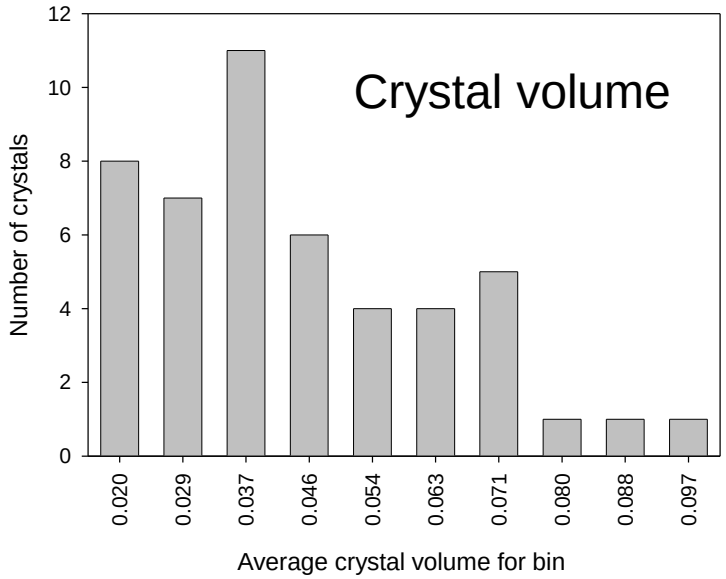


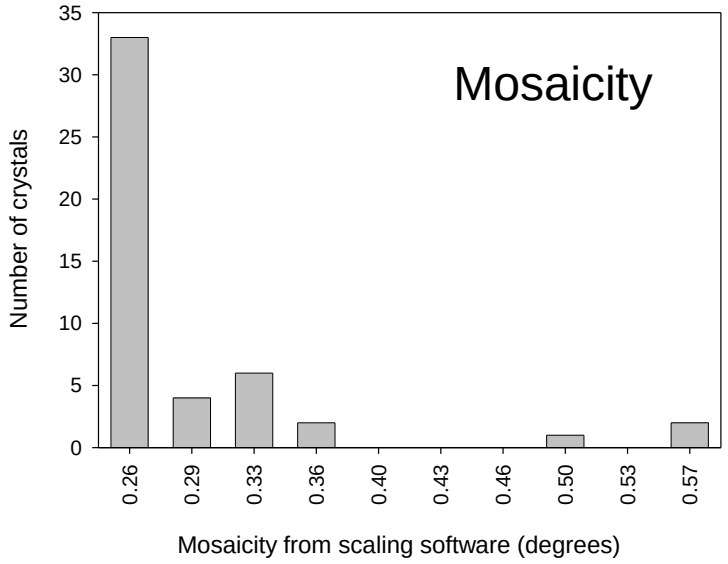
- Mosaicity from
  - Crystal volume
  - Domain volume
  - Domain alignment
  - Lattice variation
- Increased mosaicity decreases the signal-to-noise of the reflection.
- Increased mosaicity also smears the reflection in reciprocal space.

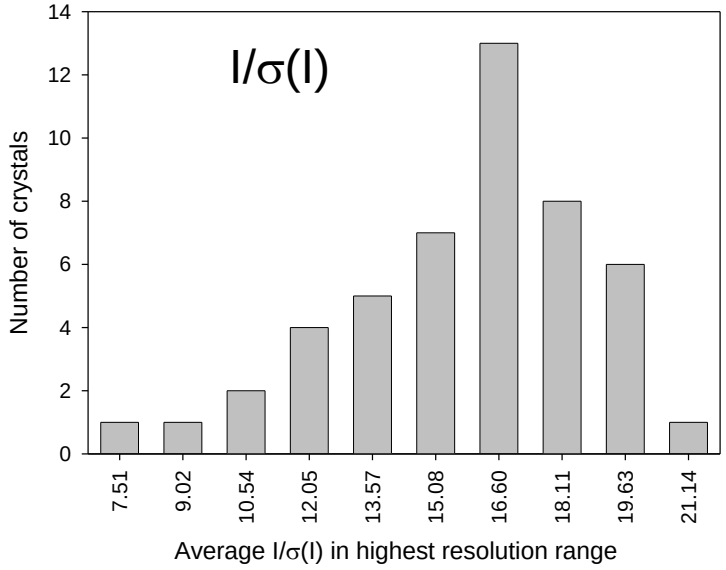
	Independent of:						Use to compare:	
Metric	Data collection protocol	X-ray beam	Detector	Qualitative judgment	Structure solution		Within same sample	Across samples
Measurable from partial or complete data								
Mosaicity (physical)	Yes	Yes	Yes	Yes	Yes		Yes	Yes
Mosaicity (software)	Yes	No	Yes	Yes	Yes		No*	No*
Resolution at defined I/σ(I)	No	No	No	Yes	Yes		No*	No*
R_{merge}	No	No	No	Yes	Yes		No*	No
Cell parameters	Yes	Yes	Yes	Yes	Yes		Yes	No*
Measurable from as complete data as possible								
Completeness	No	Yes	No	Yes	Yes		Yes	No*
Multiplicity	No	Yes	No	Yes	Yes		Yes	No*
B_{factor}	No	No	No	Yes	Yes		Yes	No
Rfactor	Yes*	Yes*	Yes*	Yes*	No		Yes	No
Rfree	Yes*	Yes*	Yes*	Yes*	No		Yes	No
Ramachandran plot	Yes	Yes	Yes	Yes	No		Yes	No
Deviations	Yes	Yes	Yes	No	No		Yes	No

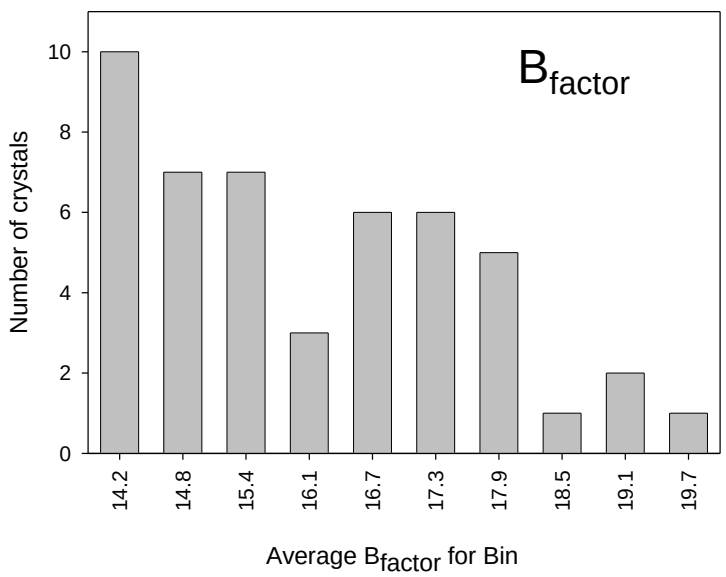
What is the variability of metrics

- Choose identical samples
  - 50 identically grown lysozyme crystals mounted individually in 0.7 mm quartz glass capillaries
- Use identical data collection parameters
  - A swathe of 80 degrees, in 40, 2 degree oscillation, 10 minute exposure collected using a CuKa rotating anode source, crystal to film distance 96 mm, collimator 0.3 mm with an Raxis IIC image plate as detector. Images processed with Denzo/Scalepack and using the CCP4 suite.
- Choose metrics:
  - Volume, unit cell parameters, mosaicity, full to partial ratio, R_{merge}, B_{factor}, $I/\sigma(I)$ for highest resolution shell, Illuminated volume.
- Experimental variation
  - Measurement errors, mounting technique, choice of crystal in drop, initial part of crystal illuminated etc.


Crystal volume
Number of crystals
0
2
4
6
8
10
12
0.020
0.029
0.037
0.046
0.054
0.063
0.071
0.080
0.088
0.097
Average crystal volume for bin


Mosaicity
Number of crystals
0
5
10
15
20
25
30
35
0.26
0.29
0.33
0.36
0.40
0.43
0.46
0.50
0.53
0.57
Mosaicity from scaling software (degrees)


I/σ(I)
Number of crystals
0
2
4
6
8
10
12
14
7.51
9.02
10.54
12.05
13.57
15.08
16.60
18.11
19.63
21.14
Average I/σ(I) in highest resolution range


B factor
Number of crystals
0
2
4
6
8
10
14.2
14.8
15.4
16.1
16.7
17.3
17.9
18.5
19.1
19.7
Average B factor for Bin

Relationships between metrics

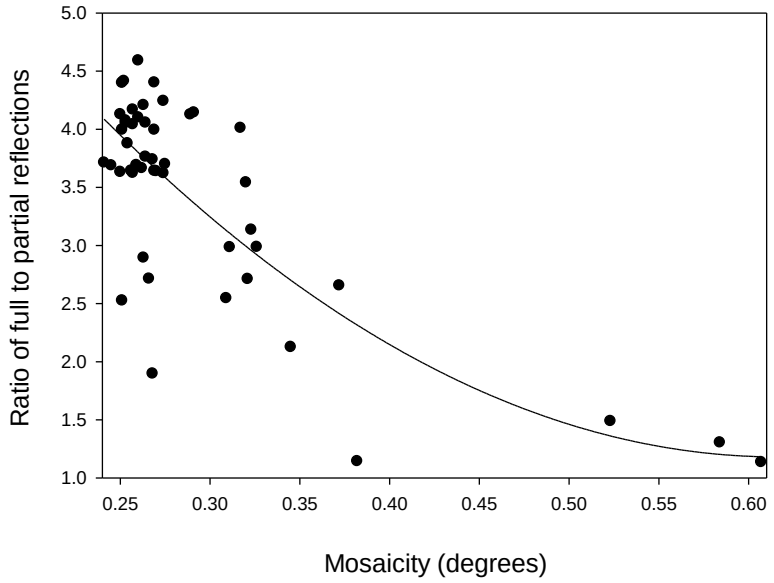
	Volume	Cell	Mosaicity	Full/partial	Rmerge	B factor	I/s(I) in highest resolution shell	Illuminated volume	
Volume	X	?	No	No	No	No	Yes	Yes	
Cell	?	X	No	?	Yes?	No	No	No	
Mosaicity	No	No	X	Yes	Yes?	No	No	No	
Full/partial	No	?	Yes	X	Yes	Yes?	No	Yes?	
Rmerge	No	Yes?	Yes?	Yes	X	No	Yes	No	
Bfactor	No	No	No	Yes?	No	X	No	No	
I/s(I)	Yes	No	No	No	Yes	No	X	Yes	
Illuminated volume	Yes	No	No	Yes?	No	No	Yes	X	

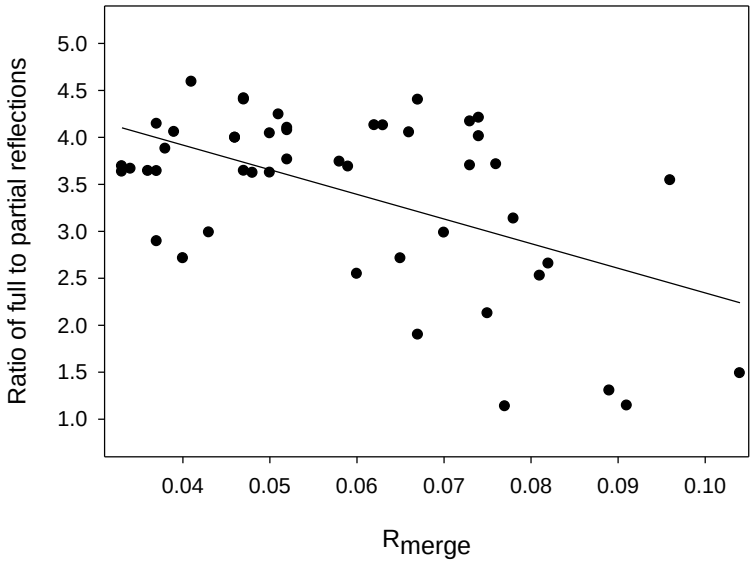
Red No – no relationship

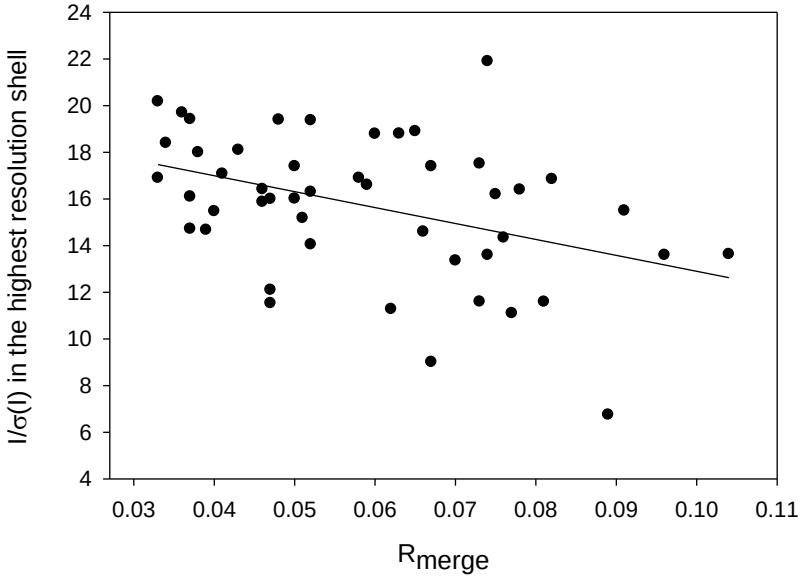
Green Yes – clear relationship.

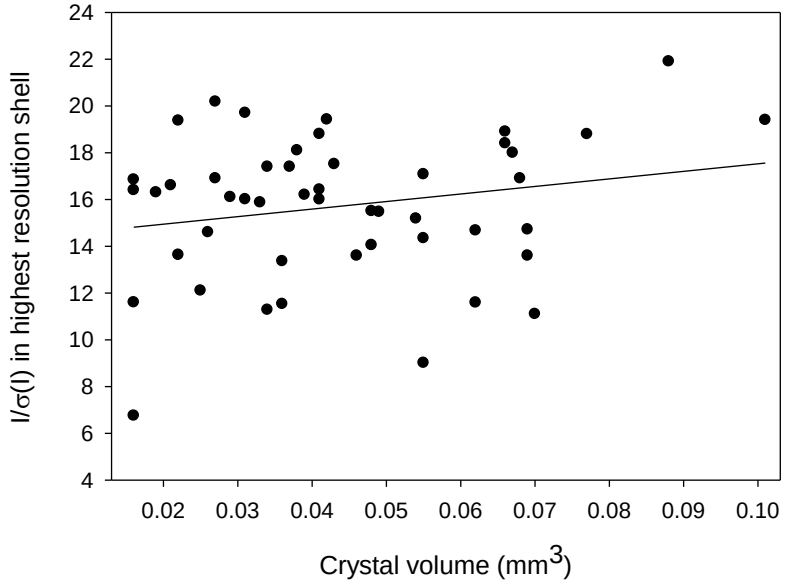
Green Yes? – looks to be a relationship but not statistically significant.

Yellow ? – looks to be a relationship but with a very high standard deviation and not statistically significant


Ratio of full to partial reflections
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
Mosaicity (degrees)


Ratio of full to partial reflections
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.04
0.05
0.06
0.07
0.08
0.09
0.10
Rmerge


I/σ(I) in the highest resolution shell
24
22
20
18
16
14
12
10
8
6
4
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
Rmerge


I/σ(I) in highest resolution shell
24
22
20
18
16
14
12
10
8
6
4
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
Crystal volume (mm3)

Identical crystals ?

- Even in identically setup samples and identical experimental protocol there can be considerable variation in the resulting data.
- There are only a few clearly related metrics:
  - Low mosaicity is related to greater full to partial ratio
  - Greater full to partial ratio is related to a lower R_{merge}
  - Increased signal-to-noise at the highest resolution is related to a lower R_{merge}
  - Increased signal-to-noise at the highest resolution is related to the crystal volume.
- There is considerable noise in the data.

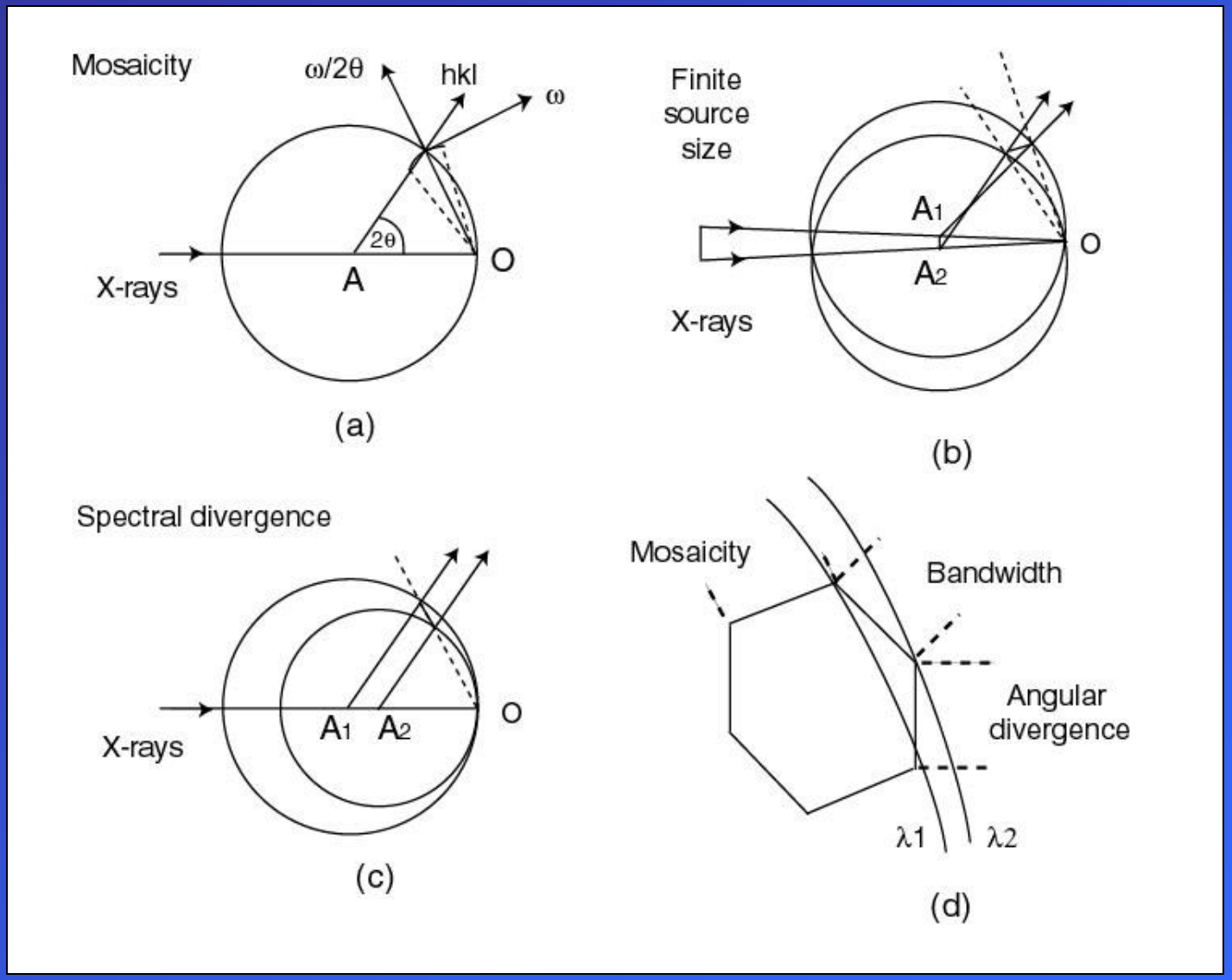
How do we identify the best?

- Reflection profile – most basic aspect of the diffraction from the crystal.
  - What part of the crystal contributes to the profile (ideally the whole crystal)
  - How is the signal manifested in the profile.

Practical aspects of measurements

- Aim - remove the experimental factors from the data and measure the underlying crystal properties.
  - Data collection protocol – Take into account exposure time, oscillation, wavelength, absorption, total data collected, processing method, software etc.
  - The X-ray beam – Take into account or eliminate variation due to intensity, spectral and geometric divergence etc.
  - The detector – Take into account detector properties etc.
  - Other – Environmental conditions etc.

Effects of some experimental factors



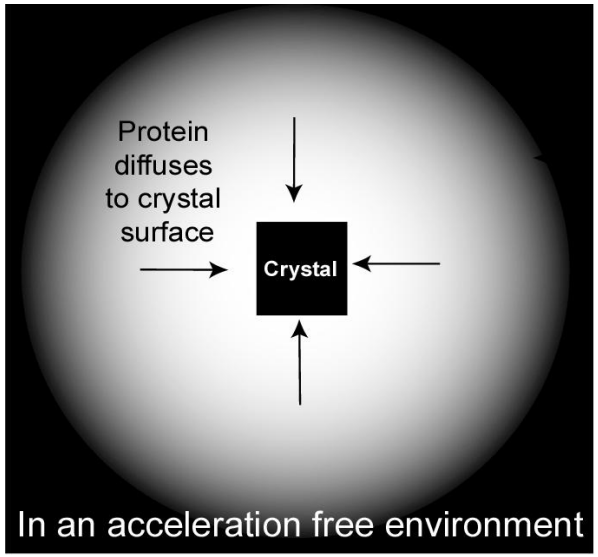
Measurements

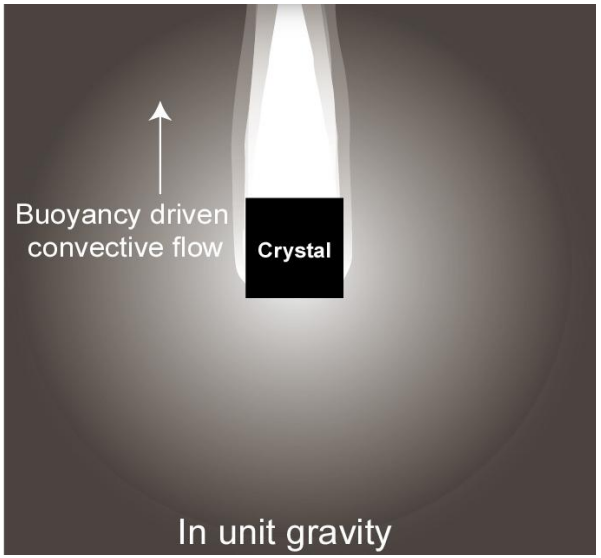
- Rapidly evaluate the crystal.
- Deconvolute effects due to the measurement and not the sample.
- Record a statistical sample of data.
- Achieve reproducibility and be able to compare with other crystals and other samples depending on the question being asked.

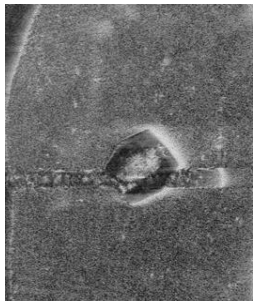
Applied to microgravity grown samples

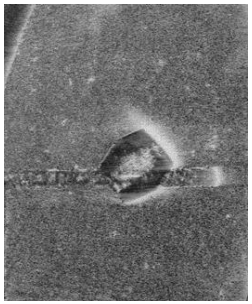
Why look at crystal quality for microgravity samples?

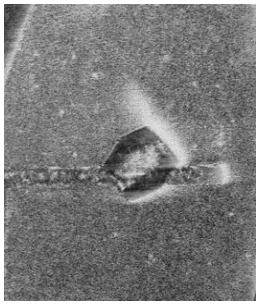
To answer the question - “Does diffusion limited growth improve the resulting crystals?”

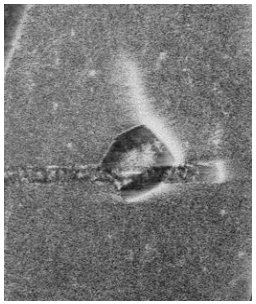


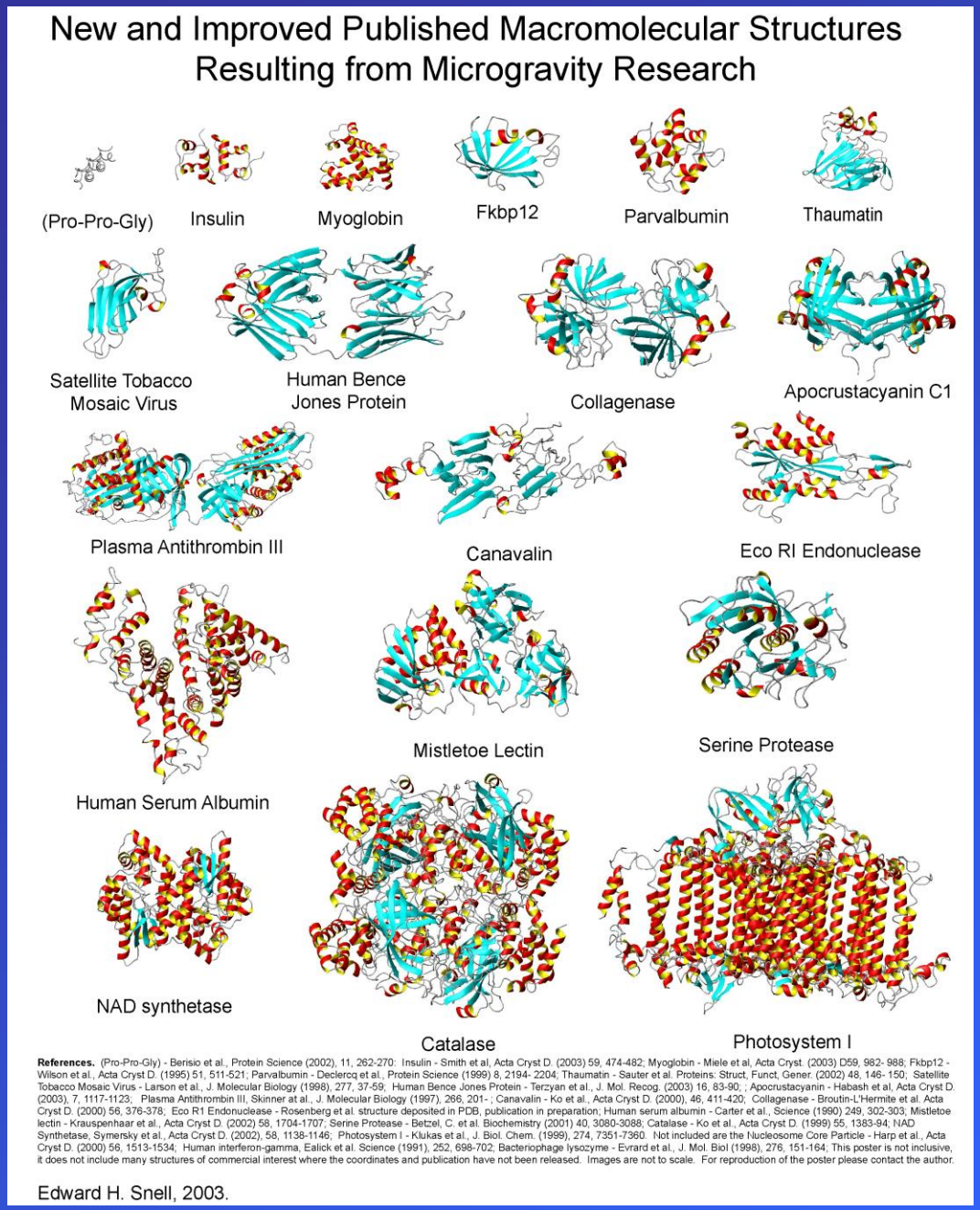












Microgravity improvement

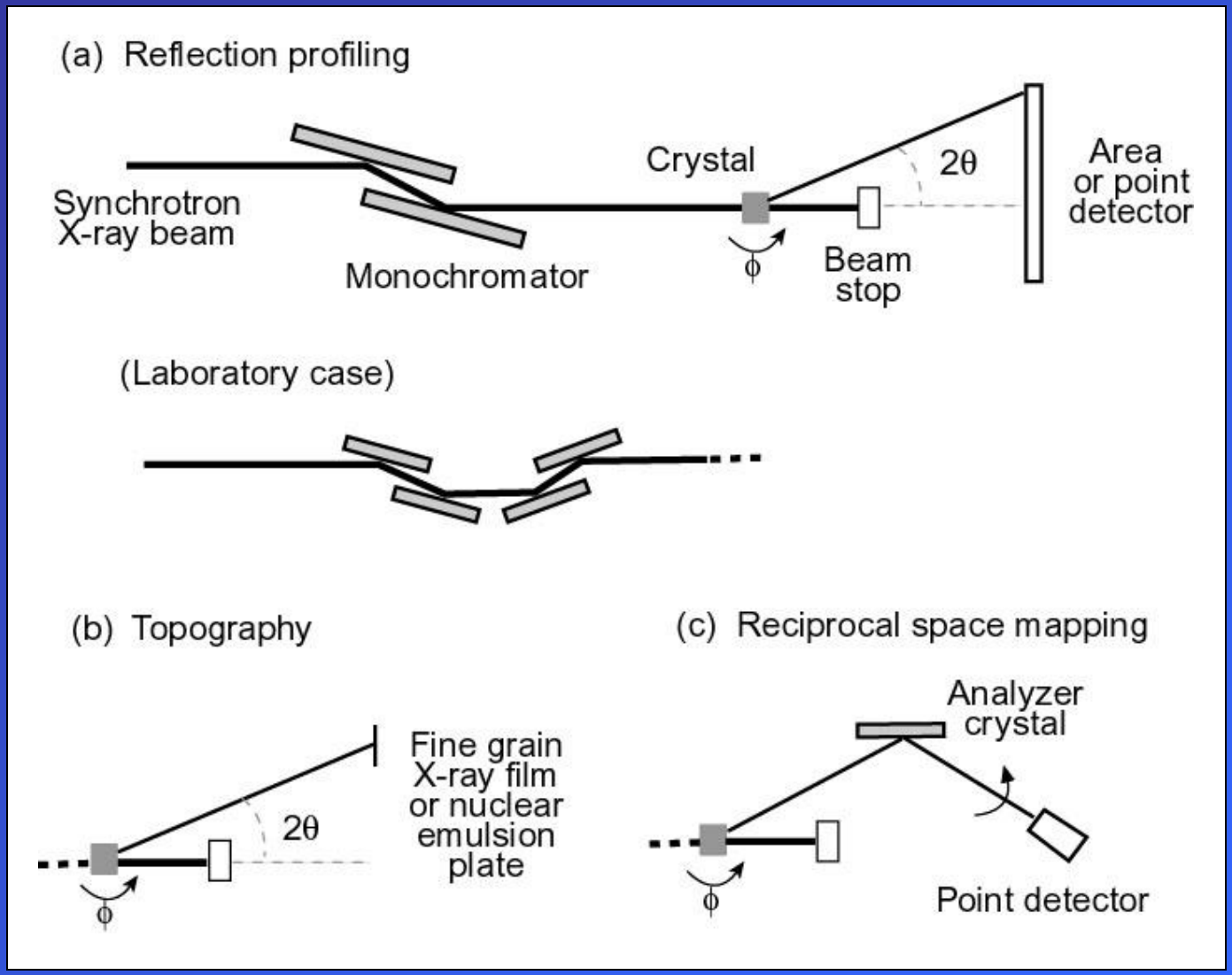
There are certainly published cases of improved structural information resulting from microgravity grown crystals.

Short-range order improves but does long-range?

Four examples studying the quality of microgravity versus ground-grown crystals

1. Careful identical data collection protocols with dose mode on the same beamline with the same dose and detector – an example with thaumatin.
2. Reflection profiling – an example with lysozyme.
3. Topography as a technique to image the whole crystal as a function of how it interacts with X-rays – an example with lysozyme.
4. Statistical reflection profiling – an example with insulin.

Experimental methods



1. Thaumatin – using standard data collection

Crystallized in the Enhanced Gaseous Nitrogen Dewar by the batch method over approx 1 month.

X-ray data was collected on a Mar 345 image plate at Stanford Synchrotron Radiation Laboratory beamline 7-1 with a 100μm^2 collimated beam.

A high resolution and low resolution data set was collected for each of 4 microgravity and 2 ground crystals.

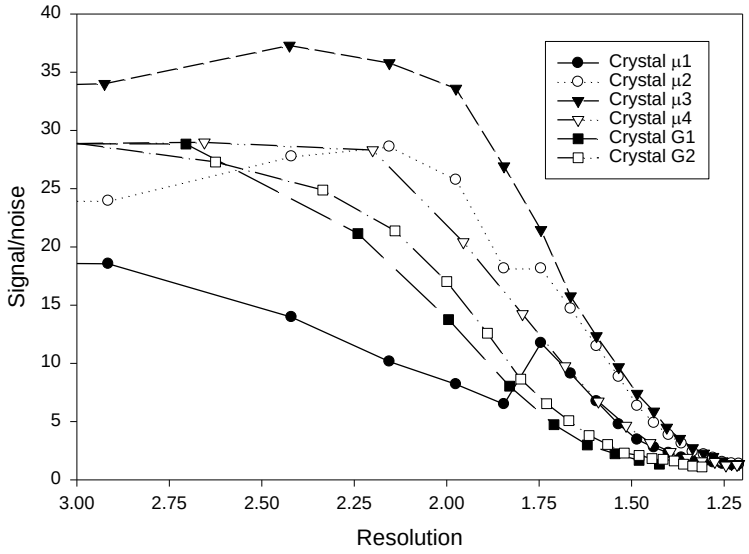
Dose mode was used to ensure that each sample received the same X-ray dose.

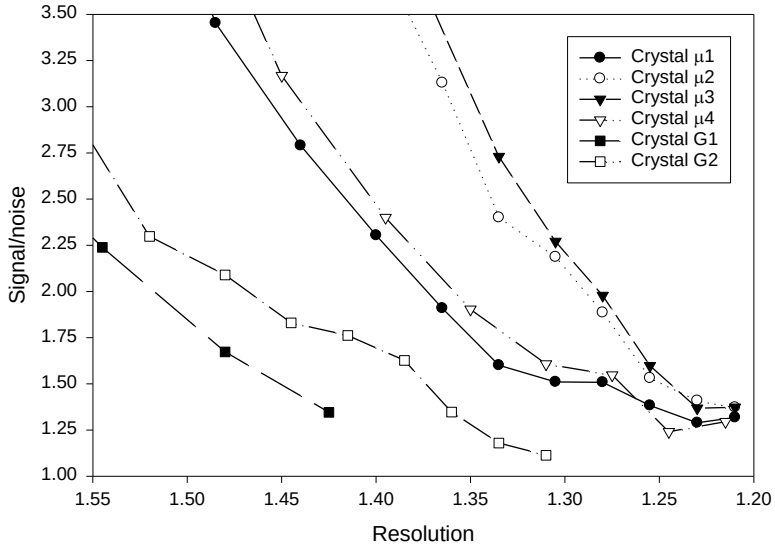
Thaumatin results

	Crystal μ1			Crystal μ2			Crystal μ3		
Size	0.97 x 0.57 x 0.46 mm			0.91 x 0.47 x 0.44 mm			1.97 x 1.00 x 1.00 mm		
Wilson Plot Scale Factor	1.34			1.18			1.00		
Wilson Plot B factor	13.0			13.4			15.3		
Cell	a=b=58.56, c=151.58 Å			a=b=58.53, c=151.59 Å			a=b=58.50, c=151.63 Å		
	Slow	Fast	Combined	Slow	Fast	Combined	Slow	Fast	Combined
Resolution (Å)	40-1.2	40-1.9	40-1.2	40-1.2	40-1.9	40-1.2	40-1.2	40-1.9	40-1.2
R-factor	9.8(60.1)	5.1(48.1)	7.4(60.1)	7.3(57.1)	5.7(13.0)	8.0(59.0)	6.8(39.0)	5.2(10.6)	6.9(39.0)
I/s	14.2(1.3)	10.7(2.2)	13.9(1.3)	17.3(1.5)	17.2(7.2)	17.5(1.4)	17.6(1.4)	23.9(11.2)	23.8(1.4)
Complete (%)	64.7(46.1)	42.7(35.6)	74.3(47.3)	85.2(74.5)	70.1(91.1)	89.3(78.2)	83.6(49.3)	86.5(98.8)	90.2(49.3)
Unique ref	54106	9270	61964	71094	15176	74394	69528	18758	75042
Mosaicity (°)	0.122	1.633	0.170	0.106	0.113	0.105	0.090	0.097	0.091

	Crystal μ4			Crystal G1			Crystal G2		
Size	0.75 x 0.34 x 0.16 mm			0.24 x 0.16 x 0.16 mm			0.34 x 0.18 x 0.18 mm		
Wilson Plot Scale Factor	1.85			4.37			3.05		
Wilson Plot B factor	13.9			15.7			14.5		
Cell	a=b=58.56c=151.37 Å			a=b=58.49, c=151.42 Å			a=b=58.52c=151.42 Å		
	Slow	Fast	Combined	Slow	Fast	Combined	Slow	Fast	Combined
Resolution (Å)	40-1.2	40-1.9	40-1.2	40-1.4	40-1.9	20-1.4	40-1.3	40-1.9	40-1.3
R-factor	4.7(39.4)	3.5(8.6)	5.9(39.3)	5.5(53.3)	10.0(85.3)	13.9(55.6)	7.4(62.9)	11.5(61.7)	15.0(62.6)
I/s	17.9(1.4)	18.4(7.2)	17.8(1.3)	16.8(1.2)	8.7(1.1)	18.6(1.3)	17.1(1.1)	11.2(1.8)	17.5(1.1)
Complete (%)	72.3(24.3)	55.0(76.6)	72.8(26.1)	89.8(62.5)	87.7(86.4)	90.7(67.3)	85.7(37.8)	91.5(98.1)	86.6(37.7)
Unique ref	60201	11916	60585	47499	18987	47897	56326	196843	56926
Mosaicity (°)	0.148	0.201	0.157	0.102	0.199	0.111	0.118	0.317	0.137

Thaumatin results





Standard structural data collection showed an improvement in diffraction resolution, signal to noise, R_{merge} and completeness for the microgravity compared to ground-grown crystals. The microgravity crystals were larger than their ground-grown counterparts (six to nine times the linear dimensions) and this is one contribution to the improvement.

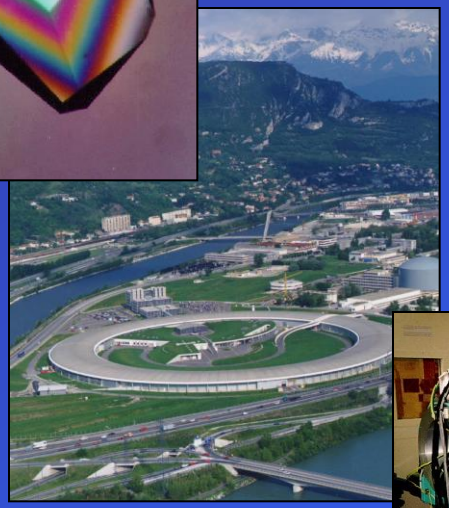
The overall B_{factor} was similar (13.0-15.7A^2) for all the crystals, microgravity and ground.

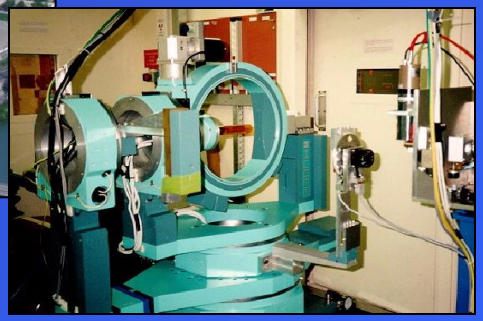
1 - Standard structural data

- Typical of that collected, i.e. a complete data set collected an area detector using a beam focused to give the maximum flux on the sample and oscillation angle appropriate for the extent of the reflections.

2-4 - Physical data from the crystals

- Single detailed reflection profiling – independent of exposure time but instrument effects need to be deconvoluted out. Not statistical unless a large number of reflections studied from different orientations.
- Topography – Gives a complete ‘picture’ of the diffraction properties of the crystal but a qualitative technique and requires very fine pixel detectors.
- Multiple reflection profiling using rapid area detectors – same advantages of single reflection profiling with the added advantage of being statistically valid.





2. Lysozyme – an old standard

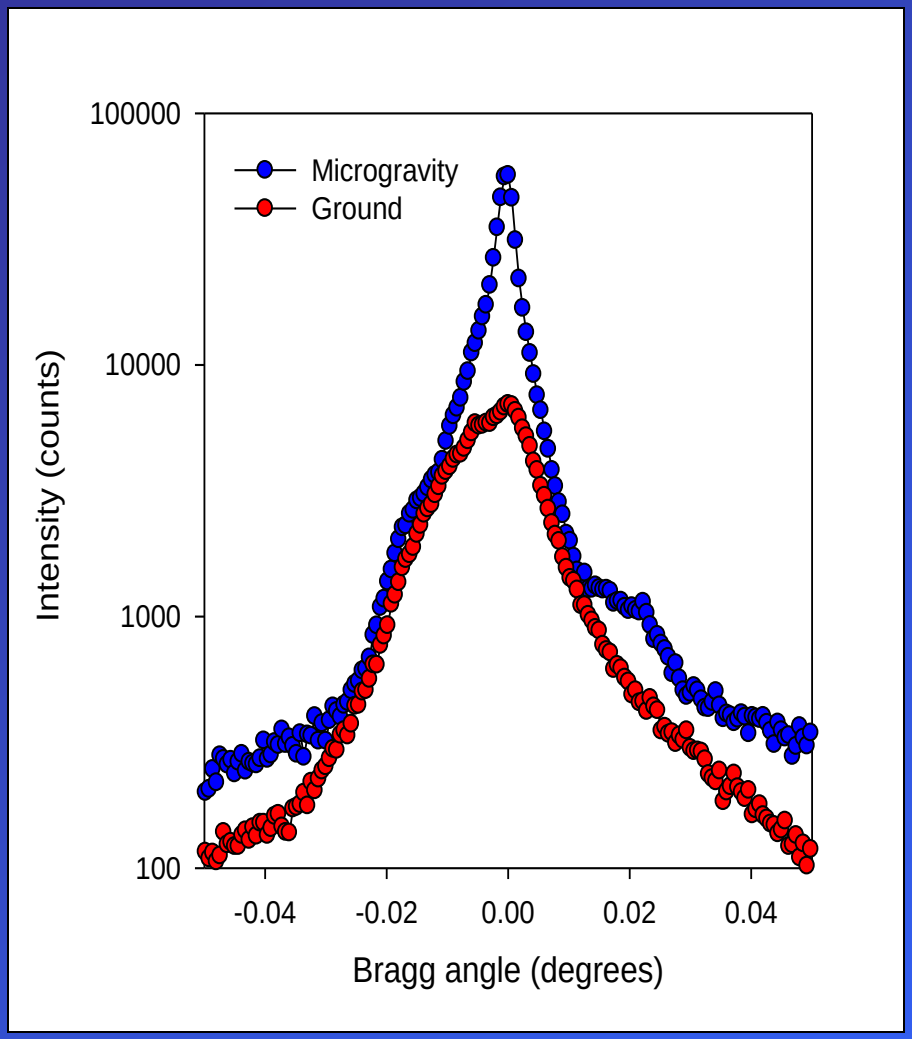
Grown in the European Space Agencies Advanced Protein Crystallization Facility on the Space Shuttle Orbiter, IML-2 and LMS missions.

Mosaicity data from room temperature crystals collected on the Swiss/Norwegian beamline of the European Synchrotron Radiation Facility, Grenoble, France.

Six circle diffractometer used for data collection.

A single reflection at a time study.

Reflection profiling



Identical reflections from microgravity and ground grown lysozyme.

Eight times increase in signal to noise.

The larger illuminated volume only accounted for a doubling.

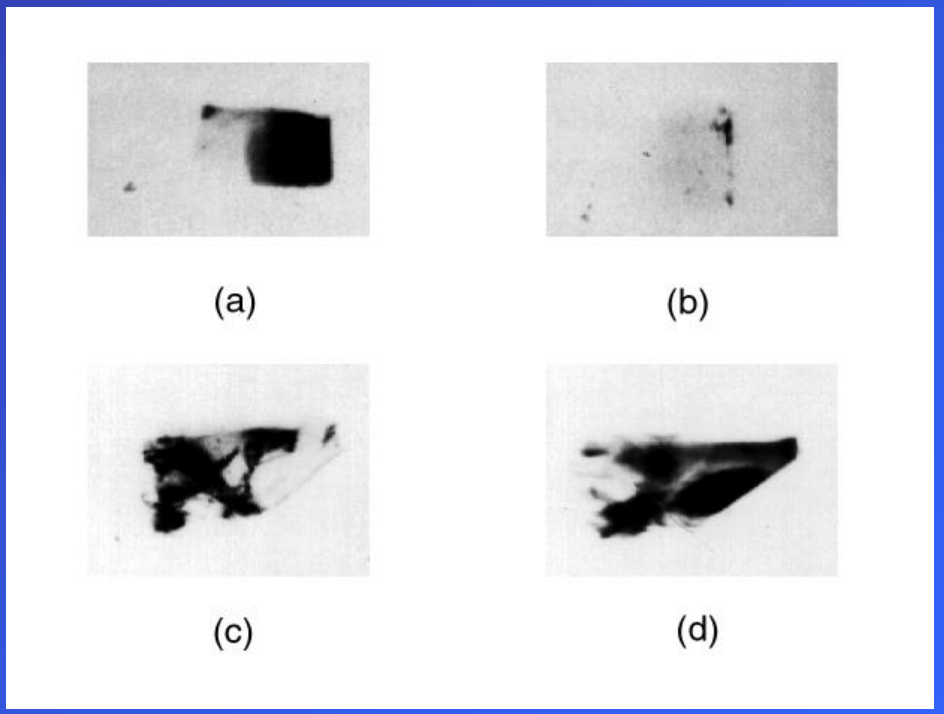
Deconvoluted mosaicity, microgravity 0.0023 degrees, ground 0.0130 degrees.

Agreement between several reflections and two crystals of each (small sample).

3. Topography

Each topograph is a greatly magnified image of a reflection.

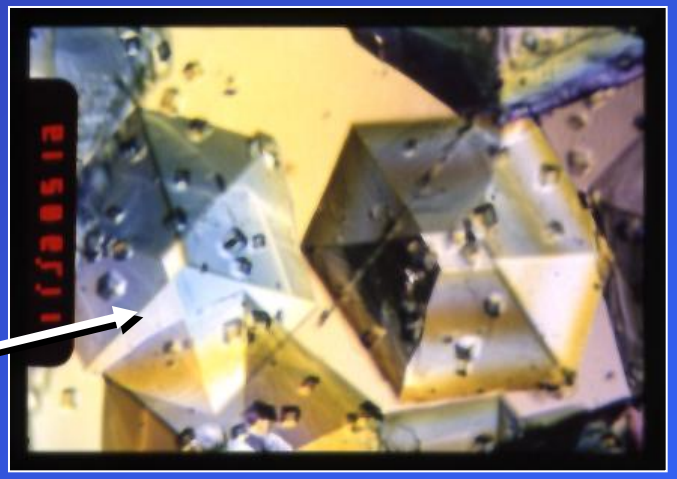
Shown are topographs from microgravity grown samples. In (a) and (b) the crystal is 1.1 mm by 0.9 mm in projection and defined regions are seen at the different reflections of (a) and (b). Some scattering is also seen on the crystal edges, probably due to mounting. In (c) and (d) the crystal is 1.5 mm by 1.1 mm in projection. In this case an array of domains is seen separated by a boundary layer. The different reflections (c) and (d) illustrate a region in the lower right of the crystal coming into the Bragg diffracting condition at the current orientation.



The topographs from the earth-grown samples are grey by comparison – they have no large regions illuminated completely at any one Bragg angle.

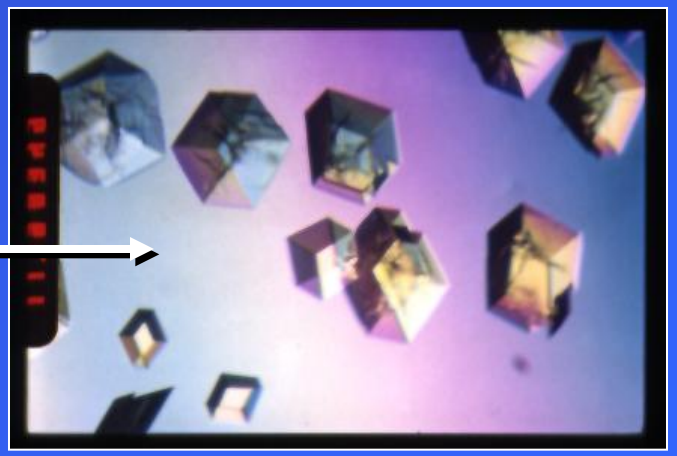
4. Insulin – Multiple reflection profiling

Grown in the Protein Crystallization Facility (PCF) by temperature reduction on the STS-95 Space Shuttle mission (Launched October 29th, 1998).



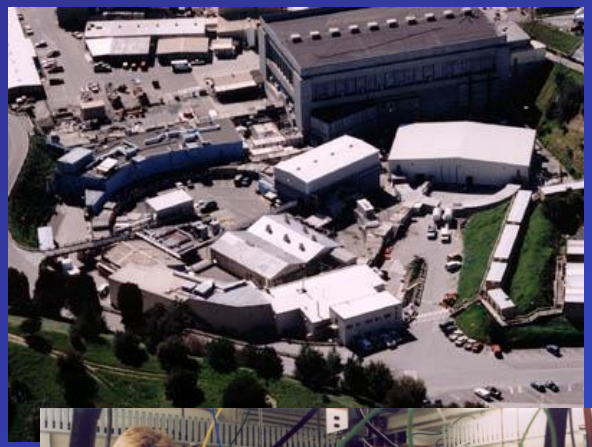
Microgravity:

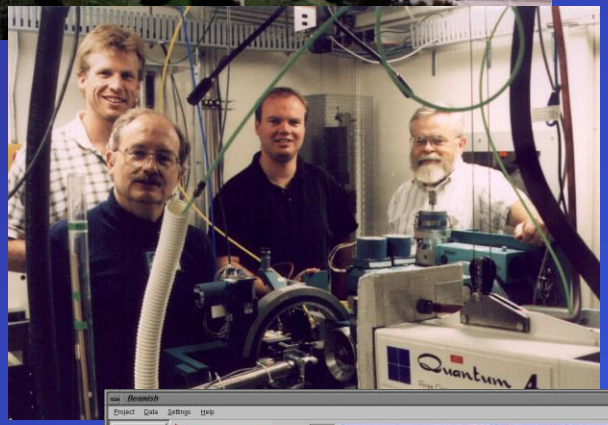
- Free floating, unsedimented. had consistently larger diffracting volume > 2 mm in each dimension (34 times larger on average)

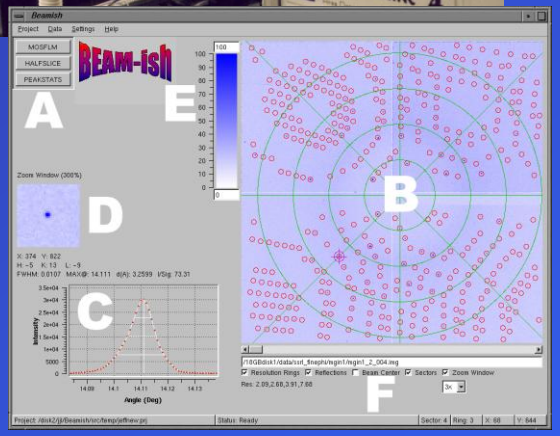


Ground:

- Sedimentation onto the bottom. Clumping of crystals.







- Data collected on beamline 1-5 of the Stanford Synchrotron Radiation Laboratory.
- Six microgravity and six earth-grown crystals were studied.
- Fine phi slicing of 0.0001 degree steps was used to collect a minimum of two 1 degree orthogonal swathes of data.
- The mosaicity was evaluated from the equation;

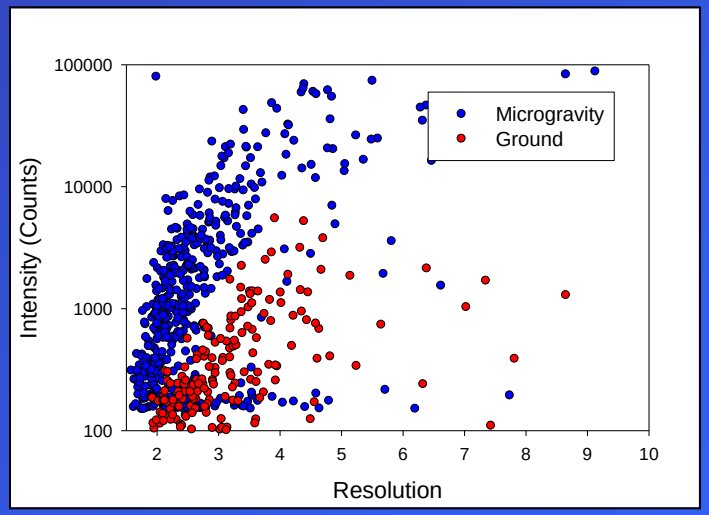
$$\eta = \frac{|\phi_R| - \sqrt{L^2\zeta^2{\gamma_H}^2 + {\gamma_V}^2}}{Ld^* \cos\theta_{hkl}} - \left(\frac{\delta\lambda}{\lambda}\right)\tan\theta_{hkl}$$

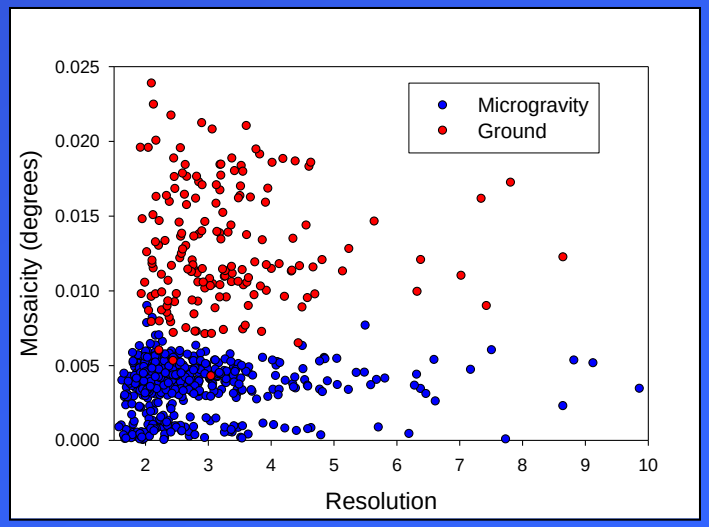
using the program Beamish.

Insulin results

Table 1 Diffraction Statistics

Sample	Date[¶]	Orthogonal crystal dimensions (mm)	Crystal Volume (mm^3)	Avg. Max. Intensity (counts)	Avg. η[#] (degrees)	No. Refl.	No. data frames
earth-grown insulin crystals[†]							
earth-1	12/98	0.35X0.35X0.32	0.04	859	0.031 (0.017)	170	2000
earth-2	12/98	0.34X0.26X0.13	0.01	880	0.035 (0.015)	20	500
earth-3	12/98	0.40X0.27X0.19	0.02	914	0.017 (0.005)	174	2000
earth-4	7/99	0.43X0.34X0.19	0.03	202	0.038 (0.024)	14	2000
earth-5	7/99	0.39X0.24X0.22	0.02	590	0.013 (0.004)	172	1999
earth-6	7/99	0.39X0.24X0.17	0.02	431	0.023 (0.010)	72	2000
microgravity-grown insulin crystals[§]							
µg-1	12/98	0.96X0.88X0.37	0.31	18776	0.004 (0.002)	502	2000
µg-2	12/98	1.20X0.72X0.48	0.42	19528	0.006 (0.005)	241	1000
µg-3	12/98	0.90X0.88X0.32	0.25	8195	0.004 (0.004)	176	500
µg-4	7/99	1.29X0.84X0.43	0.47	12846	0.002 (0.001)	491	2000
µg-5	7/99	1.72X1.31X0.90	2.04	8362	0.004 (0.002)	489	2000
µg-6	7/99	1.59X1.59X0.50	1.25	7155	0.003 (0.001)	447	2000





Worst microgravity and best ground-grown crystal illustrated in the graphs.

Common results for microgravity crystals

- Long-range
  - Mosaicity is decreased when the instrumentation is sensitive to measure the decrease.
  - Volume is increased in all cases.
  - Signal-to-noise increases in all cases.
  - Topography shows large single domains contributing to the reflection at any single Bragg angle for microgravity crystals.
- Short-range
  - Signal to noise is increased
  - There is not sufficient in these examples to say what other improvements occur.
  - Other studies have produced improved structural information – resolution enhanced or other factors coming into play?

What is the best crystal overall?

- One that provides the necessary structural data, is physically as perfect as possible and visually looks good.
- Structurally?
  - High short-range order will result in the best diffraction data.
- Physically?
  - Low mosaicity means good signal-to-noise and a reduction in errors from any partial summation, Lorentz correction etc.
- Why visually?
  - Nice for the publication (but not an essential requirement for quality)

- If a new crystal gives improved knowledge over an existing crystal then the quality has improved.

Conclusion

- There is no single metric that individually defines a high quality crystal.
- There are good qualitative and quantifiable indicators of long-range order.
- Indicators of short-range order require extensive consideration of the experiment parameters for comparison purposes.
- Quality is defined based on the question asked - If the data from the crystal allows the question to be answered then the crystal is of high quality.
- In this case beauty is in the eye of the inquisitor.

Acknowledgements

- Laboratory based studies: Russell Judge
- Thaumatin: Craig Kundrot and Cindy Barnes
- Lysozyme mosaicity: John Helliwell, Edgar Weckert, Susan Weisgerber
- Lysozyme topography: Titus Boggon, John Helliwell, Peter Siddons, Vivian Stojanof.
- Insulin: Gloria Borgstahl, Jeff Lovelace, Walt Pangborn, Dave Smith.
- Others: Staff at the University of Alabama in Birmingham, ESA, NASA, ESRF, NSLS, SSRL.
- Funding from NASA.